NOTES ON THE

Preparation of Microscopical Sections of Teeth and Bone.

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Notes on the Preparation of Microscopical Sections of Teeth and Bone.

By J. HOWARD MUMMERY, M.R.C.S., L.D.S.

Mr. President and Gentlemen,—My communication to the Society to-night will be chiefly of a technical nature, but I think it may be interesting to many members to introduce to them a method of preparing sections of teeth and bone which has not been generally in use in this country, and which may prove of considerable service in studying the histology and pathology of the teeth.

I also propose to exhibit on the screen some photographs of specimens prepared in this way, demonstrating certain points in dental structure, which it especially well brings out. We have all of us gone through the tedious process of cutting dry sections of teeth, and found it, in a measure, unsatisfactory, for we have the tissue in a dried-up condition, having procured, in fact, but the preparation of a skeleton—the soft parts having entirely disappeared, and the relations of the dentine and cementum to the pulp and peridental membrane being entirely lost.

Although such dry sections are instructive,

there is a sharp limit to their usefulness, and some other method must be resorted to if we wish to study the relations of the pulp, and its odontoblast layer, to the formed dentine, and of the cells and tissue of the peridental membrane to the cementum.

Prior to the commencement of calcification, of course the ordinary methods employed in the histological study of the soft tissues of the body, are fully available, but when calcification has commenced, the unequal degree of hardness of the tissues renders the ordinary method useless.

To study a developing tooth in which calcification has commenced, it is necessary to decalcify the portion already impregnated with lime salts; to remove these latter by the action of an acid.

Although much has been, and can be done with these decalcified specimens, they also have serious drawbacks.

It is very difficult to cut sections of these preparations with the microtome, without displacing the decalcified tissue from the pulp and peridental membrane; a few happy specimens only, among a large number of sections, exhibiting the soft parts in contact with the dentine and cementum, and we rarely procure a very thin section available for the higher powers of the microscope by this method. But there are other drawbacks to the process—we do not know ex-

actly what alterations may be produced by the acid employed, both in the decalcified portion and in the cell elements.

As pointed out by Dr. Black in his work on *The Periosteum and Peridental Membrane*, the action of the acids "is injurious in a large degree, and robs the tissues of that freshness so necessary to the gaining of good views of their constituents." He also points out that the finer chemical relations of the tissues, rendering them susceptible to delicate stains, are often disturbed by the acids used, and selective staining rendered impossible.

Being struck with the imperfections of the usual methods of preparing tooth sections, and disappointed with my own results, I was interested by a suggestion made to me by Mr. Tomes some two years ago.

Professor Moseley of Oxford had mentioned to him a plan of hardening sections of teeth and bone by gradually increasing strengths of alcohol, and then impregnating them gradually with a solution of dried Canada balsam in chloroform, but giving no detailed account of the process.

In the *Journal of the Royal Microscopical Society* for December, 1888 (p. 1042), an extract was published from the *Zeitschrift für Wissenschaftliche Mikroskopie*, giving a detailed method of carrying out this balsam process by Dr. L. A. Weil.

I prepared some sections according to these directions, and was so pleased with the results that I have since cut nearly two hundred specimens in this way. By employing this process no decalcification is required, and the cells and connective tissue of the pulp and also of the peridental membrane are retained in their natural relations to the hard tissues.

To quote from the extract in the Microscopical Society's *Journal*:—

"Dr. L. A. Weil takes only fresh, or nearly fresh teeth, and in order to allow reagents and stains to penetrate into the pulp cavity, divides the tooth immediately after extraction with a sharp fret saw, below the neck, into two or three pieces, allowing water to trickle over it the while."

To procure longitudinal sections it is advisable to cut them a little to one side of the pulp cavity, just opening this enough to enable stains to penetrate.

"The pieces are then laid in concentrated sublimate solution to fix the soft parts." The advantage of the sublimate appears to be due to its coagulating the albumen of the tissues—it certainly seems to be very efficacious in preventing shrinkage.

"The sections are then washed in running water for about an hour, and placed in 30 per

cent. spirit for twelve hours, and for a corresponding period in 50 per cent. and in 70 per cent. spirit.

"To remove the black sublimate precipitate the teeth are then laid for twelve hours in 90 per cent. spirit, to which 1·5 to 2·0 per cent. of tincture of iodine has been added. The iodine is removed by immersion in absolute alcohol until the teeth become white.

"They are now ready for staining, and the stain which Dr. Weil recommends is borax carmine (alcoholic or aqueous solutions). After being washed for fifteen to thirty minutes in running water, they are left in the stain for two or three days; they are then transferred to acidulated 70 per cent. spirit (70 per cent. spirit, 100 ccm., muriatic acid 1 cm.), in which they remain—the watery stained ones at least twelve, the alcoholic stained ones twenty-four to thirty-six, hours. They are then immersed for fifteen minutes in 90 per cent. spirit and then for half-an-hour in absolute alcohol, after which they are transferred to some etherial oil for twelve or more hours.

"The etherial oil is quickly washed off with pure zylol, and they are placed for twenty-four hours in pure chloroform; after this they are passed into a solution of balsam in chloroform.

"This balsam is prepared by drying in a water bath heated gradually up to 90° C. for

eight hours or more, until when cold the balsam will crack like glass on being punctured."

Much trouble may be saved by procuring this desiccated balsam ready prepared.

"The sections are allowed to lie for twenty-four hours in a thin solution of this dried balsam in chloroform, and then as much balsam is added as the chloroform will take up. The sections covered with the balsam solution are then placed in a suitable receptacle over a water bath, kept at 90° C., and this cooking kept up until the mass of balsam, with the teeth in, cracks like glass when cold. This requires two or three days.

"Thin pieces are then cut from them with a sharp fret saw, under water, and they are then ground down" (first on a corundum wheel, afterwards on a stone) "in the usual manner."

My most successful sections have been ground down on a washita stone, using a piece of cork, or the finger, and plenty of water.

The *débris* can be very conveniently washed off the completed section with a fine spray of water blown through an ether spray apparatus. The section is then mounted in chloroform balsam.

The process, as detailed, no doubt appears very tedious and complicated, and it is almost enough to deter anyone who has but little leisure

from undertaking it, but when a number of sections are being prepared in different stages, the passing on from one solution to another does not occupy much time.

Wolrab's gold bottles in a rack form excellent receptacles for the sections, a note being made on a label on the bottle, of the stage they have reached.

With this, as with most other processes, there are of course a good many failures, some being caused by insufficient cooking, the pulp not being sufficiently hardened; too prolonged cooking, on the other hand, is apt to cause brittleness.

The cutting down is certainly very tedious and must be done on a slow cutting stone; rapid cutting, as with a turkey stone, I found resulted in the pulp being crowded with small particles of the stone, which adhere firmly to the balsam, and I know of no means of getting rid of them.

Of course, too, without great care, in grinding very thin sections the pulp may break away at the last moment, and it is only by practice one can learn to avoid this annoying accident.

The stain recommended by Dr. Weil—borax carmine—penetrates well, and stains the nuclei very strongly, but does not give so much detail in the pulp as some other stains. Very good results may be obtained with aniline blue black, which stains the nerve fibres as well as the

nuclei and connective tissue. I have not been very successful with hæmatoxylin, but am told that Ehrlich's hæmatoxylin, which does not precipitate, would probably be the best stain to use in this process.

I must here express my indebtedness to Mr. Theodore Harris for the great help he has given me in preparing these sections; his assistance has been invaluable in carrying through most carefully the tedious preliminary processes.

The teeth I have made use of have been chiefly young bicuspids, some with the apex of the root still incomplete—extracted for regulating purposes, and I take this opportunity of thanking several friends who have sent me specimens.

I have also made sections of older teeth for comparison, and of some carious teeth and abscesses. In these latter I think the process might prove very useful—enabling one to study the early stages of abscess formation.

Mr. Swift has been kind enough to attend with his projection microscope, by means of which he will be able to project upon the screen the actual preparation on the stage of the microscope. With this instrument we are limited to low-power objectives, as with higher powers there would be too great a diminution of light.

The transparencies presently to be shown are necessary to exhibit the *details* of the speci-

mens, but the slides now shown with a one-and-a-half inch objective, will serve to indicate some of the advantages of the process—as the retention of the pulp in its natural relations to the dentine, and the absorbent cells in temporary teeth occupying the excavations in the tissue, and in the slide showing the rat's molars, it will demonstrate that without decalcification we can exhibit the teeth in their natural relations to the surrounding bone.

The slide showing a longitudinal section of a molar tooth, which has been the subject of severe attrition, shows a secondary deposit in the pulp, surrounded by a transparent zone in the dentine, opposite the surface which has been exposed to the greatest wear.

The transparency No. 1. shows a transverse section of the pulp of a bicuspid tooth. It exhibits the pulp with its relations to the walls of the pulp cavity apparently undisturbed. The odontoblast layer is seen (very distinctly differentiated from the rest of the pulp) lying in immediate contact with the semi-calcified portion of the dentine—the tissue "on the borderland of calcification," that part of the matrix which has evidently undergone some change, in advance of the line of complete calcification. The blood-vessels are seen in transverse section, and also the slightly denser condition of the

central part of the pulp, noticeable in many of these specimens. (Plate I., Fig. 1.)

The next slide from a similar pulp, is interesting as showing, in the large blood-vessels in the centre, what very delicate tissue can be retained in position by the hardened balsam during the process of grinding.

The next slide shows a portion of the pulp and the forming dentine, taken with a half-inch objective and magnified eighty diameters. A blood-vessel is involved in the line of odontoblasts; the semi-calcified portion of the dentine is well seen, and the rounded masses of the lime salts marking the line of complete calcification. (Plate II., Fig. 1.) These rounded masses are still better seen in the next slide, from a longitudinal section at the margin of the pulp cavity —the coalescence of the globules to form the fully calcified tissue being very clearly shown. (Plate III., Fig. 1.)

The next photograph shows a transverse section from one of the cornua of the pulp of a bicuspid tooth, showing chiefly connective tissue and small cell nuclei, and apparently no true odontoblast layer.

The next slide is taken from a tooth which was extracted before the apex of the root was completed. The portion photographed is close to the open end. (Plate II., Fig. 2.)

The odontoblast cells, which, together with their nuclei, have taken the stain deeply, are seen not to be lying in close contact, but to have distinct spaces between them. I do not think this is due to shrinking in preparation, as I have found it in all the open-ended bicuspid teeth I have examined, and other specimens prepared by this process seem to indicate that there is no appreciable shrinkage of the odontoblast cell. Mr. Hopewell Smith, in a paper published in the *Dental Record* for August, 1889, speaking of the dentine after the commencement of calcification, says:—"Between some of the cells of the membrana eboris there are wide visible spaces, filled with homogeneous substance and small round and angular cells." Mr. Tomes also appears to have somewhat modified his views on this point, for whereas in the earlier editions of his "Dental Anatomy" he says (p. 159, second edition)—"The odontoblasts are fitted closely together, and there is no room for any other tissue between them so long as the formation of dentine is actively going on;" in the third edition the words are—"There is not much room for any other tissue between them" (third edition, p. 169). The squareness of the cells towards the forming dentine is very evident in this specimen.

The next slides show the applicability of this

process to the study of the peridental membrane. This photograph, taken with a one-sixteenth Powell and Leland, × 500, is from the margin of the cementum in a transverse section of a bicuspid tooth, stained with aniline blue black. The outer and more recently formed portion of the cementum has taken the stain strongly, and exhibits with great clearness the penetrating fibres of Sharpey,—connective tissue fibres from the peridental membrane passing deeply into the hard tissue, which in this portion seems to be chiefly made up of them. Between these bundles of fibres, where they enter the cementum, are seen the large cementoblast cells concerned in the formation of the tissue.

Dr. Black ("Periosteum and Peridental Membrane," p. 102) describes these cells as being always flattened, with one of their flat sides resting upon the cementum, and of very irregular outline, and considers that in ordinary sections we only see a profile view of them. Their true shape is, he considers, seen in sections of the peridental membrane taken parallel to the surface of the cementum.

The appearances presented by some specimens I have prepared by this process indicate something in the development of dentine not quite in keeping with the ordinary views.

There appear to be processes of the connec-

tive tissue of the pulp, adherent to the dentine, very like the penetrating fibres of Sharpey in bone. I have not yet completed my observations on this point, being at present engaged in investigating other varieties of dentine for this purpose.

In the next photograph (Plate III., Fig. 2) also from a transverse section of a bicuspid, a line of little nests of round cells is seen lying among the fibres of the peridental membrane. These agglomerations of cells lie at a little distance from the surface of the cementum, and are usually seen in young teeth, when the sections are sufficiently thin. They vary considerably in size and in the number of cells composing them. Dr. Black considers them to be lymphatics; he describes them as being more numerous near the margin of the gum, and in sections of the peridental membrane, which he has cut parallel to the surface of the cementum, at such a distance as to include them, he finds that these apparently isolated bodies are connected by a network. The groups of cells seem to be enveloped in a very delicate limiting membrane, which is, I think, visible in the next slide (taken with a one-sixteenth objective, × 500). He looks upon this structure as "lymph canals packed with lymphoid cells," rather than as true lymphatic glands.

In a case of suppurative pericementitis he found the suppuration running along the lines of these lymphatic chains to a great distance, suggesting that this tissue may be the seat of the disease.

Malassez does not consider them to be lymphatics, but the remnants of the enamel organ which extended beyond the region of forming enamel in the early stages of development.

We will now pass to some examples of absorption. The next slide shows a temporary molar in longitudinal section, with the absorbent organ *in situ*—the cells filling up the excavations in the dentine. Magnified 170 diameters. This preparation shows, perhaps almost better than any other, the advantages of the process I have described. (Plate I., Fig. 2.)

It is very difficult by ordinary methods to obtain a thin section of dentine with these absorbing cells in position, such preparations being usually quite fragmentary and the result of happy accidents. It appears to show plainly that the gradual increase in the strength of the alcohol and the preliminary coagulation with the sublimate solution prevents shrinking, as these cells completely fill the lacunæ or excavations in the dentine. This was one of the first preparations I made in this manner, and I have looked upon it as one of the test slides of the process.

In the parts of this preparation where the groups of cells are prolonged deeply into the dentine, the individual cells are large and rounded in outline. In other parts where the excavations are not so deep, ordinary multinucleated giant-cells are seen lying in contact with the dentine.

There seems to be still a good deal to be studied in absorption of the temporary teeth—the method of action of these cells being little understood. Whatever substance these cells secrete does not seem to produce any softening action on the tissue much beyond the point of contact—the excavations being clean cut and distinct.

Passing to absorption in adult teeth: as in bone, so in the cementum of healthy teeth, tooth absorption and deposition go hand in hand—many young, and to all appearance healthy, teeth, showing absorption spaces filled with giant cells, and old excavations filled up with newly-deposited material.

The photograph now on the screen is from a bicuspid extracted at fifteen years of age, and a distinct absorption is seen in the cementum, the excavation being filled up with large cells similar to those seen in the absorbing temporary tooth.

The next slide, taken from a longitudinal section of a molar in which a small piece of the bone of the alveolus remained attached to the tooth,

shows the bone on one side and the cementum on the other, the peridental membrane and periosteum filling the interval between them. There appears to have been here considerable excavation of the alveolar bone, and also a large absorption of the cementum, which has been filled up with freshly deposited tissue—the repair being actually in progress in this case—the cementoblasts being crowded together on the newly-formed cementum, as the osteoblasts are on the surface of depositing bone.

Deposits of secondary dentine in the pulp are well exhibited by this method of preparation. The specimen shown was taken from a molar tooth, to all appearance sound, which caused intense neuralgia, rendering it necessary to extract it. The pulp was densely packed with secondary deposits, encroaching in every direction upon the nerves and blood-vessels. This deposit exhibits some curious concentric and radiating masses. The next slide is from a similar pulp, showing some very large deposits.

Another shows a tooth extracted from an old person, in which the whole of the pulp appears to be converted into a semi-calcified material, apparently of cartilaginous consistency, with islands of calcified tubular dentine. I have been struck with the fact pointed out by Mr. Salter in his "Dental Pathology"—that many young and appa-

rently healthy pulps show numerous deposits of secondary dentine. Mr. Salter in the work referred to (p. 139) says: "This change is to a great extent reparative and the result of trivial causes, though I believe it never occurs unless the tooth has been in some way the subject of injury or irritation."

The specimens in which I have seen it were certainly untouched by caries; but they may have been subjected to some form of irritation conveyed to the pulp from the great pressure caused by overcrowding.

Interglobular spaces in dentine are very well stained in the balsam process.

Caries in a fissure in the enamel.—This slide was prepared to show that the process, while keeping the relations of the carious portion to the calcified tissue, retains also in position tissue that has undergone a very considerable amount of disintegration.

The last slide is taken from a rat's incisor, and shows the remarkably strong connective tissue fibres of the pulp.

In conclusion, gentlemen, I have to thank Mr. Swift for so kindly giving his valuable personal attention to the microscope and lantern, and to thank you for your kind attention; expressing the hope that some of the points I have simply touched on to-night may suggest lines of investigation to those engaged in microscopical work.

Weil's Process.

Fresh teeth cut under water with watch-spring saw.
Concentrated corrosive sublimate solution for some hours.
Running water one hour or more.
30 per cent. spirit, twelve hours.
50 per cent. spirit, twelve hours.
70 per cent. spirit, twelve hours.
90 per cent. spirit, + 2 per cent. iodine, twelve hours.
Absolute alcohol till teeth are white.
Running water half an hour.
Stain borax carmine, &c., three to seven days according to stain used.
70 per cent. spirit (+ 1 per cent. h.c.l. if borax carmine) twelve to thirty-six hours.
90 per cent. spirit, fifteen minutes.
Absolute alcohol half-an-hour.
Etherial oil, twelve hours.
Wash this off with xylol.
Chloroform, twenty-four hours.
Thin solution of dried Canada balsam in chloroform.
Thick solution of dried Canada balsam in chloroform.
Water bath at 90° C. till hard.

Discussion.

The President said they were greatly indebted to Mr. Howard Mummery for his very lucid and able paper, and for the beautiful illustrations, and invited comments upon the paper.

Mr. F. Newland-Pedley said he was able to confirm, from his own experience, the value of the process which Mr. Mummery had described, but like many other things it was of some antiquity, being known for the last fifteen years. Some five or six years ago one of his colleagues at Guy's wished to investigate the development of "rider's bone," and it was necessary to show hard and soft structures at the same time. He (Mr. Pedley) cut sections by Mr. Mummery's method, and they were shown at the Pathological Society.

Mr. H. Baldwin wished to ask Mr. Mummery whether the transparent portion which intervened between the dark layers was not chiefly formed of the unstained portion of the odontoblast cell, and whether the dark layer did not consist of the nuclei only of the odontoblast? Also, whether the so-called spaces were not unstained portions of the cells? Again, as to the small agglomerations of cells which were found in the periosteum, and which were said by some to be epithelial pearls, whether there was not some stain which would show whether they were of an epithelial nature or not?

Mr. Arthur Underwood said if it would not be taking a liberty to relieve Mr. Mummery of the trouble, he would take upon himself to answer two of the questions. If the parts were stained with gold the whole cell becomes perfectly stained, and the interspaces are quite marked, and that without confusion of substances; still more the transparent layer with lines between the cells is marked out quite plainly, being in no kind of sense a part of the cell—there could be

no kind of confusion—there was no doubt that an interval does exist. Mr. Underwood thought that they ought really to feel very much indebted to Mr. Mummery for his epoch-making paper in dental microscopy. Though Mr. Pedley seemed to have been so happy as to have hit upon the same process a long time ago, still it had not become public property until that evening. All dental microscopists would feel under a great debt of gratitude to Mr. Mummery for having solved the difficult problem how to cut hard and soft tissues together, leaving them undisturbed by the influences either of the knife or the fluids. Mr. Underwood knew from experience how ready critics were to assert that the appearances were due to decalcifying fluids. He thought a tablet should be raised to Mr. Mummery for having delivered them from these tiresome critics.

Mr. Charles S. Tomes wished in endorsing Mr. Underwood's remarks to emphasise the fact that Mr. Mummery had been the first to produce preparations which would go very far towards necessitating a revision of much that had been said and written on the question of the development of dentine. The points in question Mr. Mummery had hardly touched upon, because until he had thoroughly worked the subject out he very rightly did not wish to say anything he might have to recede from. Mr. Tomes would not have said anything about it had not Mr. Mummery confined himself very much to the exhibition of the process, and had not Mr. Pedley said that the process was not very new, thereby implying that it was not worth while demonstrating what the process could do. This much he would assure Mr. Pedley that Mr. Mummery's preparations were entirely novel to him (Mr. Tomes); they showed things in a manner which he had never seen approached, and he felt sure they would give some results which would necessitate a great deal of re-writing.

Mr. George Cunningham desired to call attention to the fact that the photographs were all the work of Mr. Mummery himself, so that he was not only an able microscopist, but also a photomicrographist who might vie with Mr. Andrew Pringle, who had been described as *facile princeps*.

Mr. Charters White felt that he ought to add his testimony in favour of Mr. Mummery's very able paper. He (Mr. White) had been reading for the last thirty years on the subject of microscopy and photomicrography, and had made the subject a special study, but he was bound to say he had never been so fortunate as to reach the process before. Decalcification of the bony tissues resulted in the destruction of the shape of the cells, and the presence of acid in the pulp makes them very difficult to stain. Mr. White felt that Mr. Mummery had given the death knell to the decalcification of the tissues, and he would for one adopt the process new to him, because he felt it was capable of giving details which decalcification had never yet afforded. He would like to ask Mr. Mummery if the sections could be rubbed down in the manner in which he (Mr. White) had always described for dry sections, because in that way it would be possible to get photographs much clearer and sharper.

Mr. W. Hern wished to ask Mr. Mummery if he could explain how it was that after a process of prolonged and powerful heating, soft tissue—which was known to contain a large percentage of water—seemed to occupy the same space?

Mr. Charters White, if he might be allowed to reply, thought that the use of the corrosive sublimate as a fixing agent, and then afterwards the hardening in absolute alcohol, prevented any further change. The soft tissue being saturated in Canada balsam, Mr. White did not see how it was possible for the real histological elements to alter.

Mr. Howard Mummery, in reply to Mr. Pedley, was not aware that the process had been really well known before, although of course there had been hints of it. To Dr. Weil he thought belonged the credit of bringing it out properly and giving all the minute details necessary for practical working. Mr. Baldwin had already been answered by Mr. Arthur Underwood so ably that Mr. Mummery felt it unnecessary to add anything upon the points which he had raised. He quite agreed with Mr. Underwood that the spaces between the odontoblast layer and the fully calcified

dentine are not part of the cells ; if Mr. Baldwin would look at the specimens under the microscope he would see that. Mr. Mummery wished to thank Mr. Tomes for his kind remarks. He quite agreed with Mr. Charters White as to the evil effects of decalcification, and thought that the specimens might be cut down by Mr. White's method, though as he was only feeling his way he had not yet adopted it. In reply to Mr. Hern, he would say that the spirit takes the place of the water, and the gradual increase in the strength of the spirit prevents shrinking of the tissues. The addition of the balsam solution in graduated strengths drives out the spirit, the balsam taking its place. The slow substitution of one reagent for another—together with the very important fixing of the fresh tissue with sublimate—is the main principle of the process.

Fig. 1.

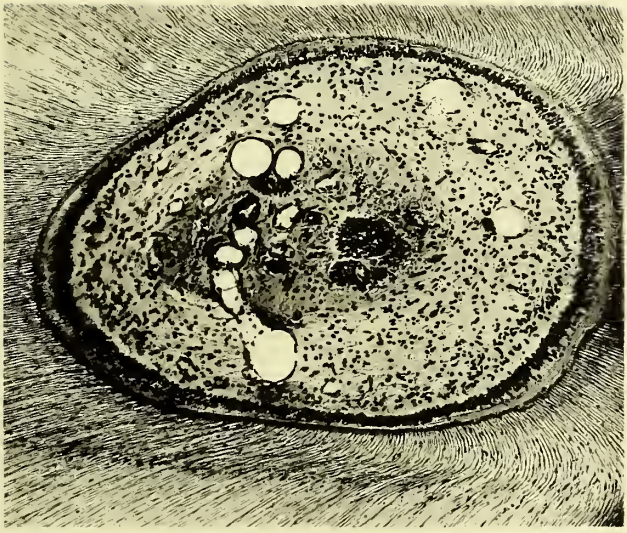

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Fig. 2.

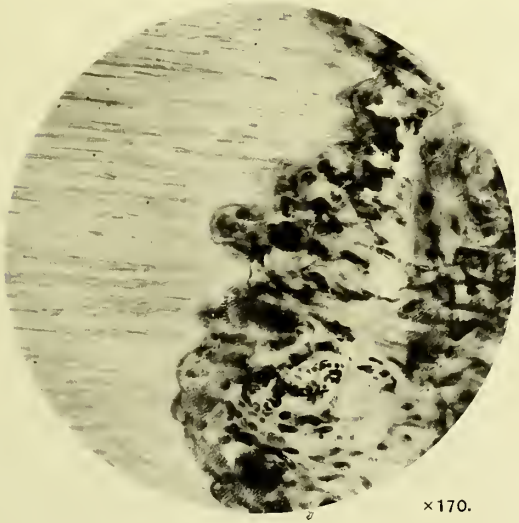

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Fig. 1.

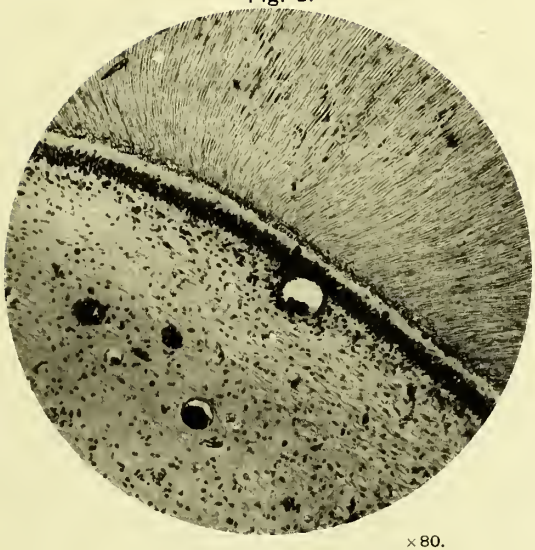

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Fig. 2.

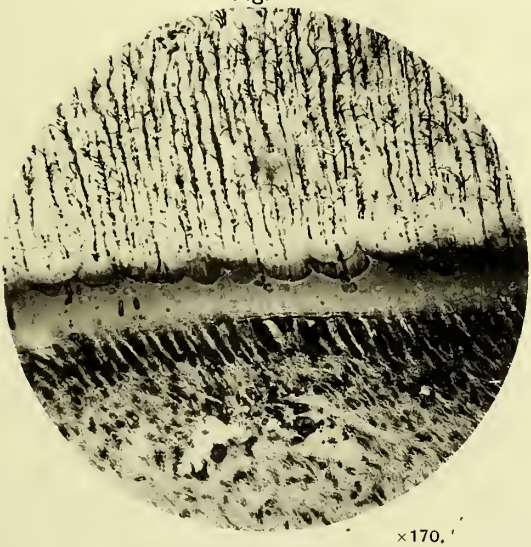

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PLATE III.

Fig. 1.

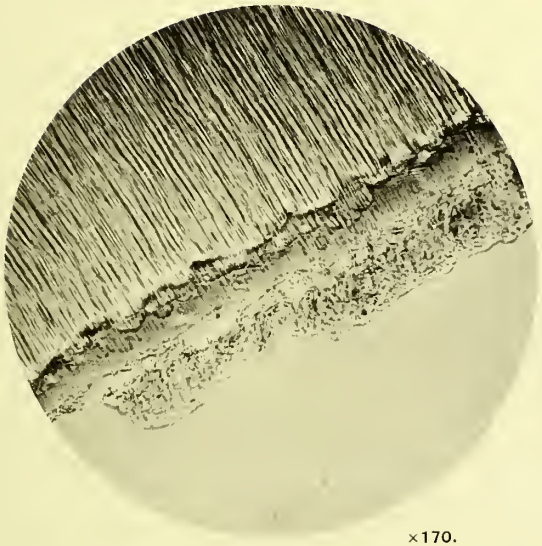

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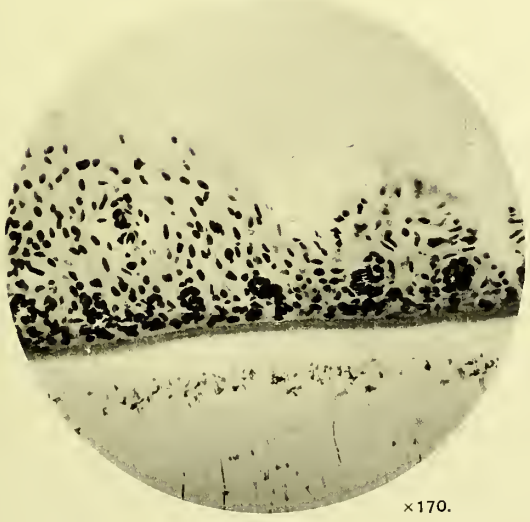
Fig. 2.



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